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☐ 1: J Biomol NMR. 2002 Sep;24(1):41-50.

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Novel projected 4D triple resonance experiments for polypeptide backbone chemical shift assignment.

Xia Y, Arrowsmith CH, Szyperski T.

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Ontario Cancer Institute and Department of Medical Biophysics, The University of Toronto, Canada.

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Here we present a novel suite of projected 4D triple-resonance NMR experiments for efficient sequential assignment of polypeptide backbone chemical shifts in $^{13}\text{C}/^{15}\text{N}$ doubly labeled proteins. In the 3D HNN[CAHA] and 3D HNN(CO)[CAHA] experiments, the $^{13}\text{C}(\alpha)$ and $^1\text{H}(\alpha)$ chemical shifts evolve in a common dimension and are simultaneously detected in quadrature. These experiments are particularly useful for the assignment of glycine-rich polypeptide segments. Appropriate setting of the ^1H radiofrequency carrier allows one to place cross peaks correlating either backbone $^{15}\text{N}/^1\text{H}(\text{N})/^{13}\text{C}(\alpha)$ or $^{15}\text{N}/^1\text{H}(\text{N})/^1\text{H}(\alpha)$ chemical shifts in separate spectral regions. Hence, peak overlap is not increased when compared with the conventional 3D HNNCA and HNN(CA)HA. 3D HNN[CAHA] and 3D HNN(CO)[CAHA] are complemented by 3D reduced-dimensionality (RD) HNN COCA and NNCACO, where $^{13}\text{C}(\alpha)$ and $^{13}\text{C}'$ chemical shifts evolve in a common dimension. The $^{13}\text{C}(\alpha)$ shift is detected in quadrature, which yields peak pairs encoding the $^{13}\text{C}'$ chemical shift in an in-phase splitting. This suite of four experiments promises to be of value for automated high-throughput NMR structure determination in structural genomics, where the requirement to independently sample many indirect dimensions in a large number of NMR experiments may prevent one from accurately adjusting NMR measurement times to spectrometer sensitivity.

PMID: 12449417 [PubMed - indexed for MEDLINE]

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